

FIG. 1A

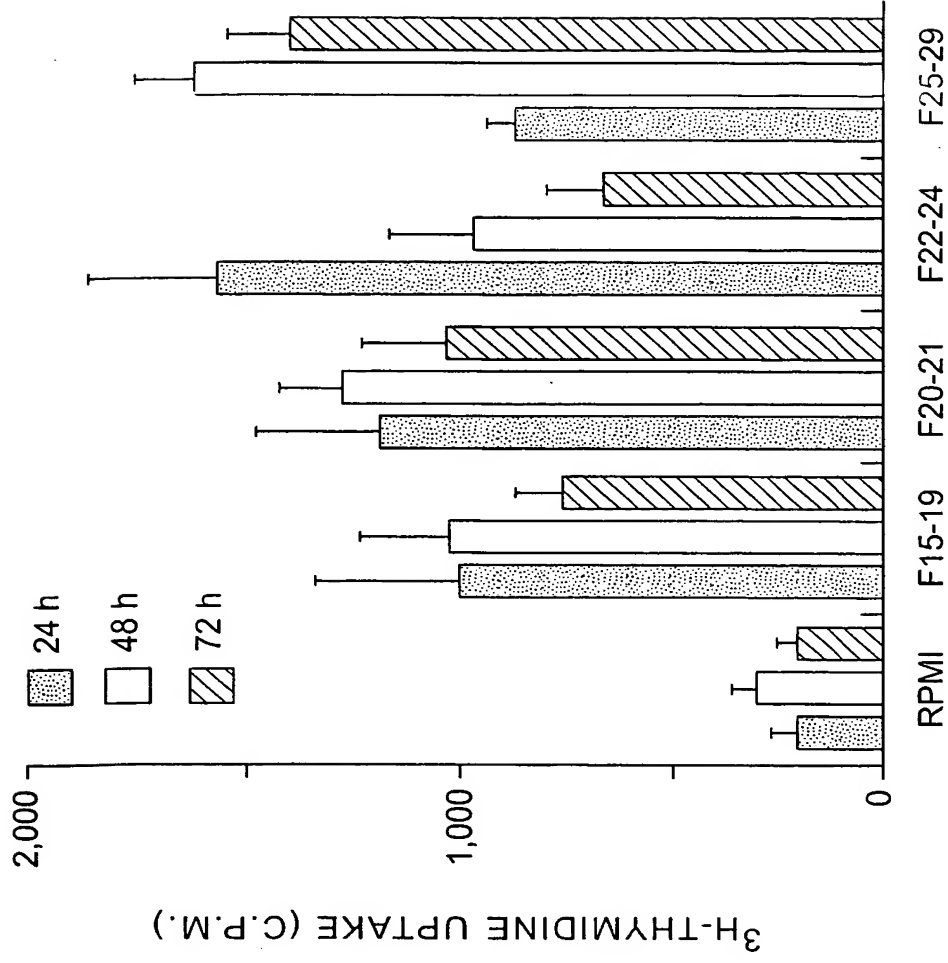


FIG. 1B

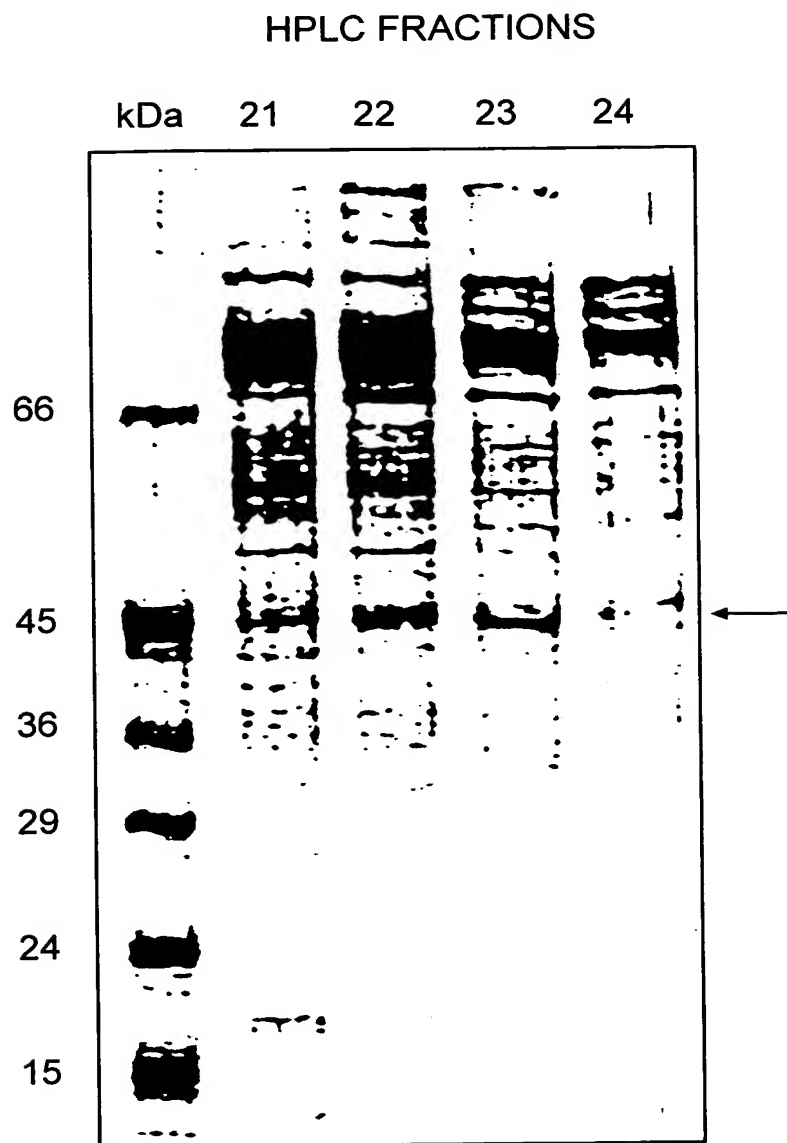


FIG. 1C

TC MRKSVCPKQKFFFSAPFFFFFCVFFPLISRTGQEKLLFDQKYKIIKGEKKEKKKNQRANRREHQKREIMREKKS 75
CS -----MKFSKG 6
Pa -----MQR 3

TC FTCDMHTGEAARIVTSGLPHIPGSNMAEKAYLQENMDYLRRGIMLEPRGHDDMFGAFLDPIEEGADLGMVF 150
CS IHAIDSHTMGEPTRIIVGGIPQINGETMADKKKYLEDNLDYVRTALMHEPRGHNDMFGSII TSSNNKEADFGIIF 81
Pa IRIIDSHTGGEPTRLVIGGFDPDLGQDMAERRRLLGERHDAWRAACILEPRGSDVLVGALLCAPVDPEACAGVIF 78

TC MDTGGYLNMCCHNSIAAVTAAVETGIVSVPAKATNPVVPVLDTPAGLVRGTAHLQSGTESEVSNASIIINVPSFLYQ 225
CS MDGGYLNMCCHGCSIGAA TVAVETGMVEMVEPTNIN--MEAPAGLIKAKVMVEN---EKVKEVSITNVPSFLYM 151
Pa FNSGYLGMCGHGTIGLVASLAHLGRIGPV-----HRIETPVGEVEATLH-----EDGSVSVRNVPAYRYR 140

TC QDVVVVLPKPYGEVRVDIAFGGNFFAIVPAEQLGIDISVQNLSRLOEAGELLRTEINRSVKVQHPOLPHINTVDC 300
CS EDAKLEVPSLNKTIITFDISFGGSFFAIIHAKELGVKVTQSQVDVLKKGIEIRDLINEKIKVQHPLEHIKTVDL 226
Pa RQVSVEVPGI-GRVSGDIAWGGNWFVLVAGH--GQRLAGDNLDALTAYTAVVQQALDD----QDIRGEDGGAIDH 208

TC VEIYGPPTNPEANYKNVVFNGNRQADR SPCGT GTSAKMATLVAKGQLRIGETFVYESILGSLFQGRV--LGEE 371
CS VEIYDEPSNPEATYKNVVFNGQVDR SPCGT GTSAKLATLYKKGHLKIDKVFYESITGTMFKGRV--LEET 297
Pa IELFAD--DPHADSRNFVLCPCGKAYDR SPCGT GTSAKLAACLAADGKLLPGQPWQRQASVIGSQFEGRYEWLDGQ 279

TC RIPGVKVPVTKDAEEGMLVVTAETITCKAFIMGFNTMLFDPTDPFKNGFTLKQ* 423
CS KVGEFD-----AIIPEITGGAYITGFNFHVIDPEDPLKYGFTV*-- 335
Pa PGGPIVPTIRGRAHVSAEATLLADDDPFAWGIRR*----- 314

FIG. 2

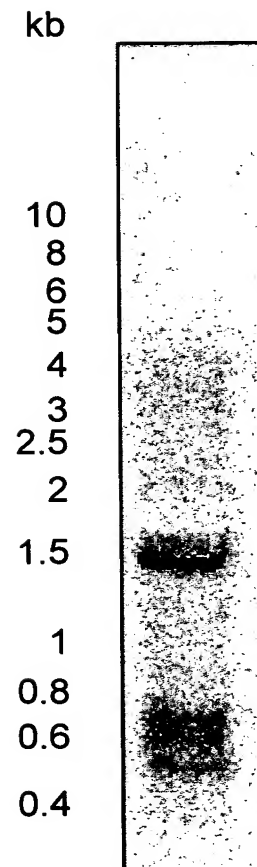


FIG. 3B

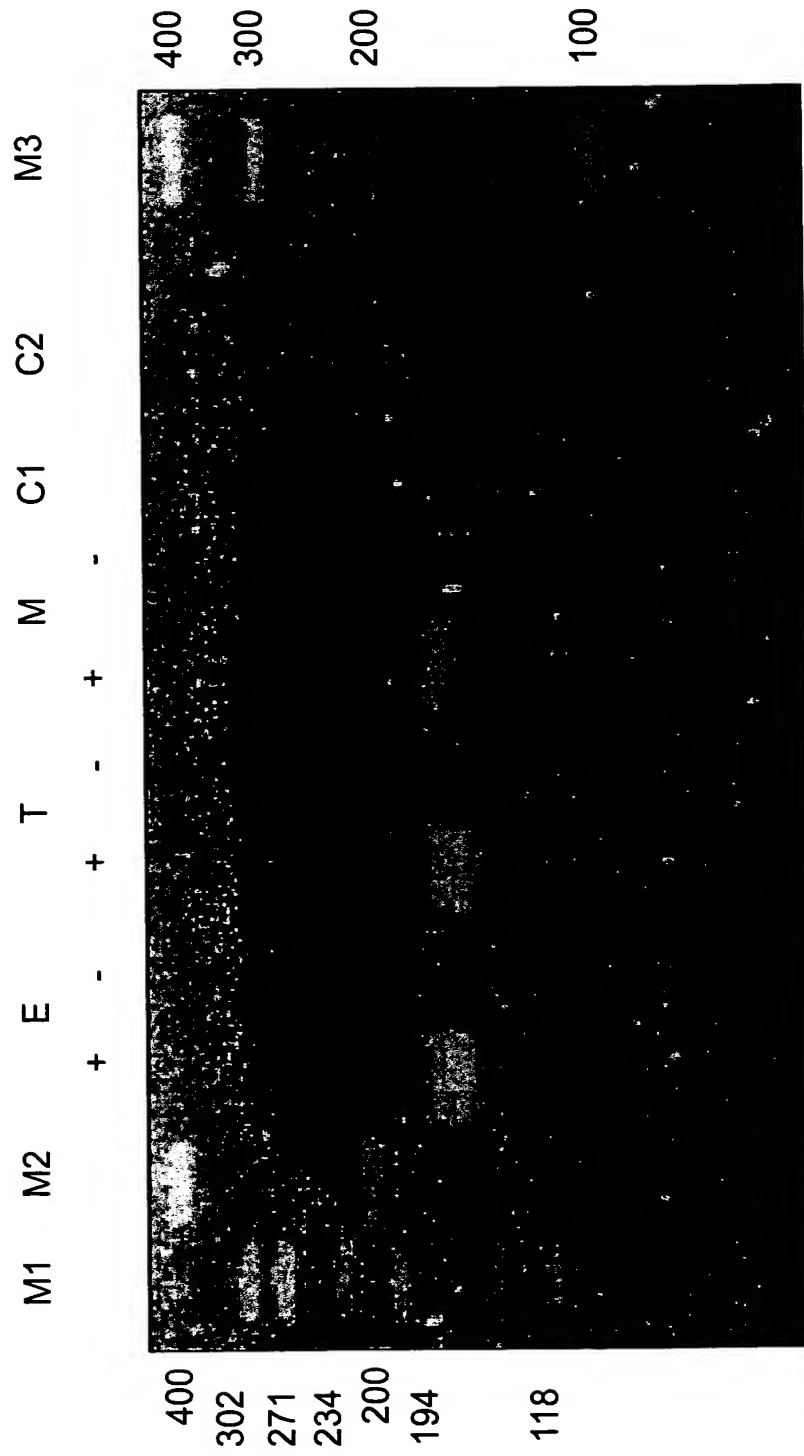


FIG. 3C

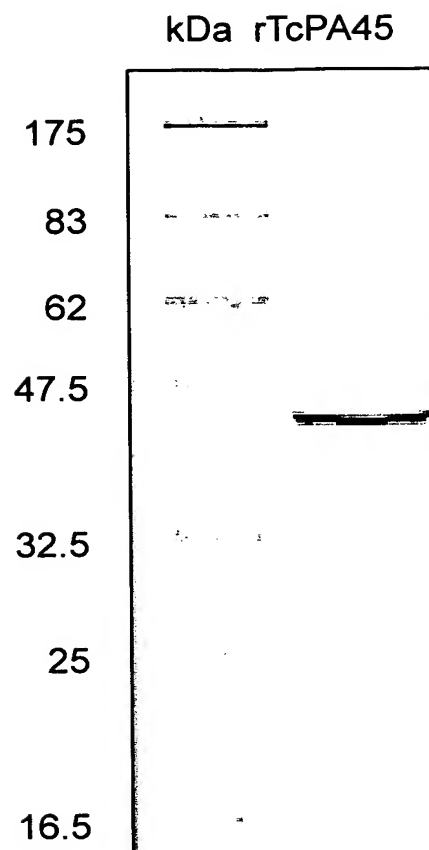


FIG. 4A

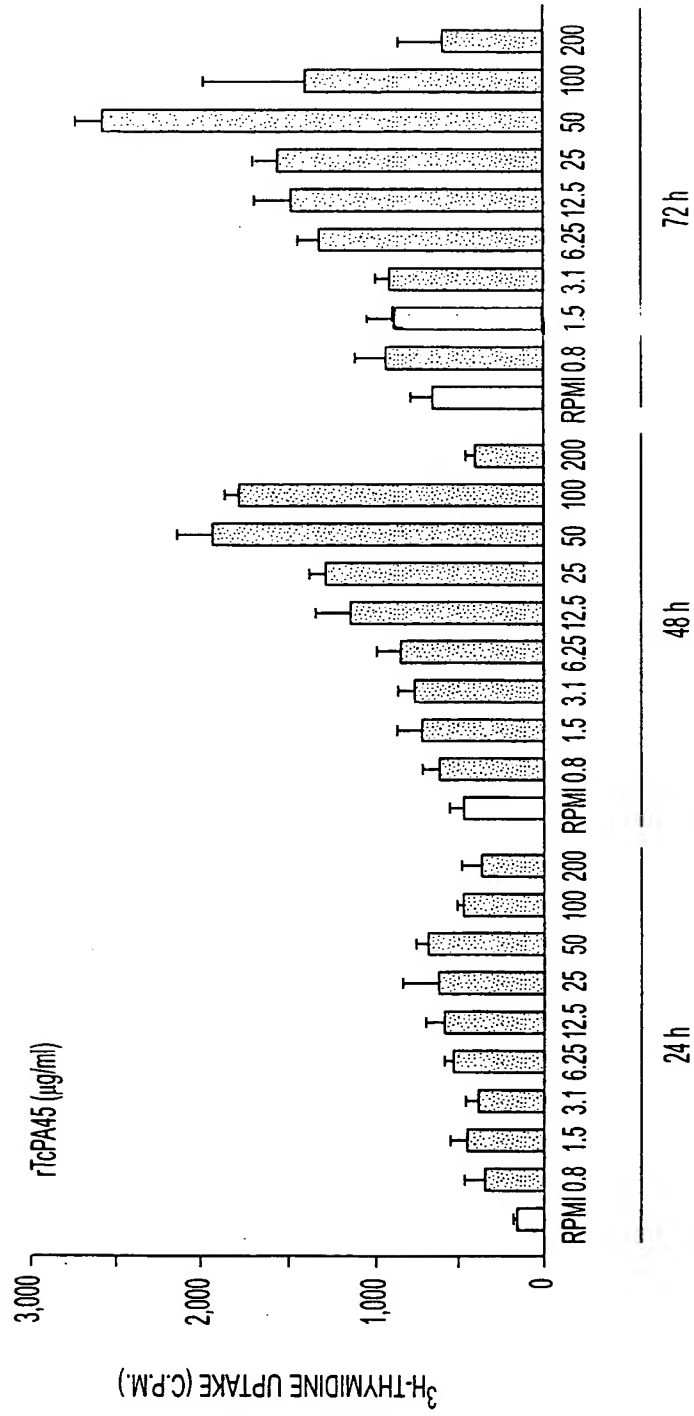


FIG. 4B

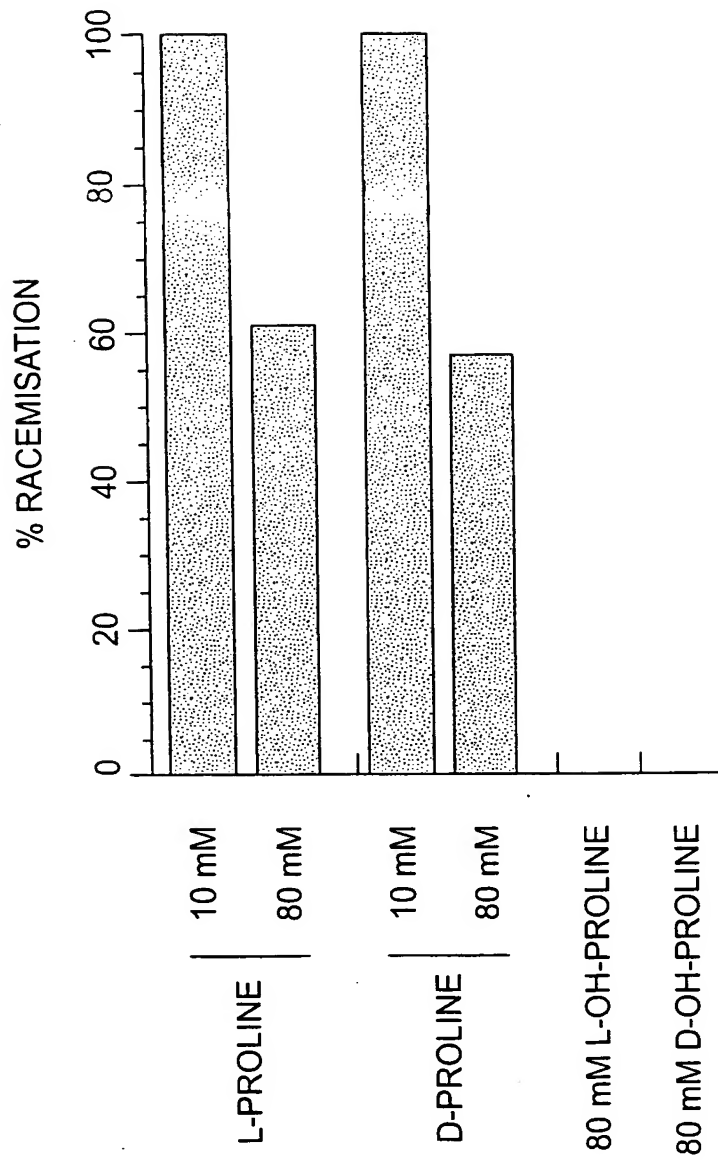


FIG. 4C

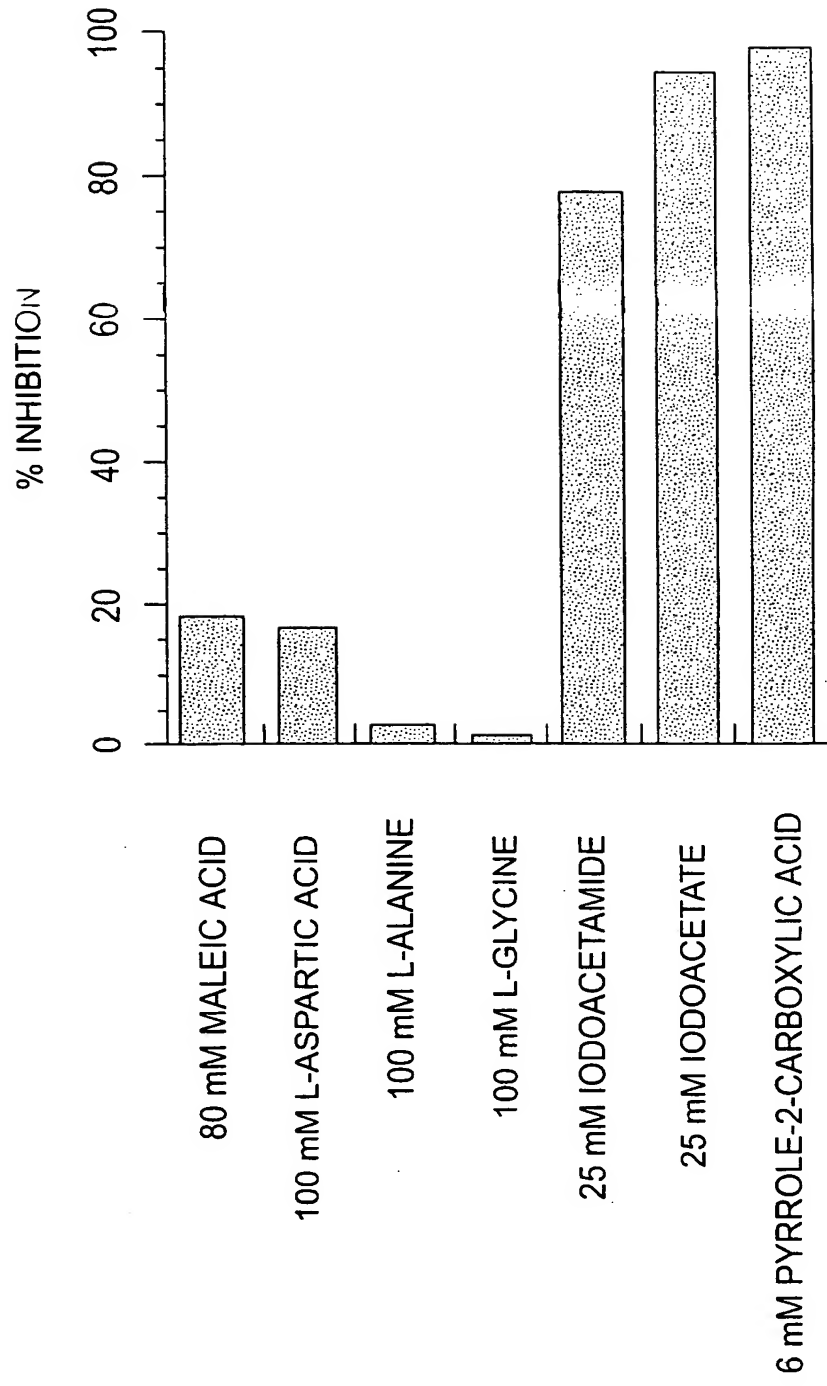


FIG. 4D

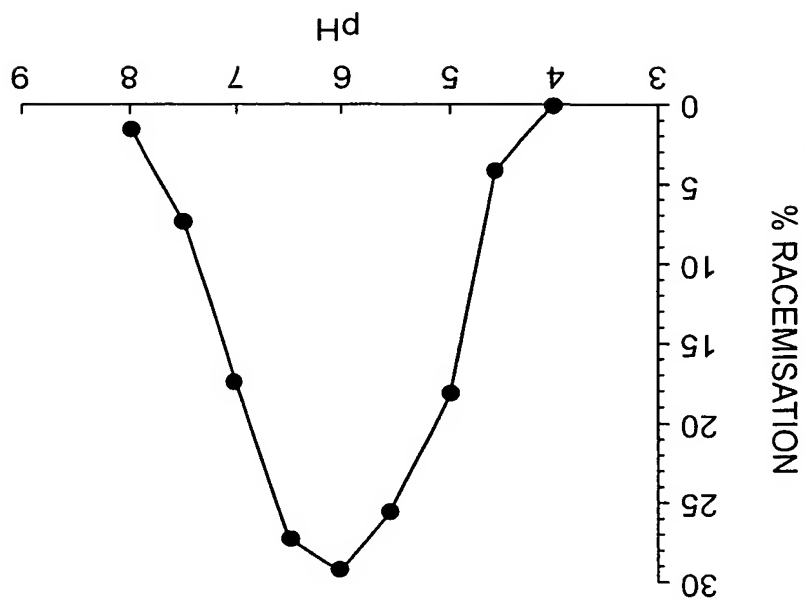


FIG. 4E

FIG. 5A

CGCAGTGTGAAGGTTACAGCACCCCTCAGCTGCCCCCATATTAACTGTGGACTGTGTGAGATATACGGTCCGCCAACGAACCCGGAGGCA 970
R S V K V Q H P Q L P H I N T V D C V E I Y G P P T N P E A 312
AACTACAAGAACGTTGTGATATTGGCAATGCCAGCGGATCGCTCTCCATGTGGACAGGCCACCGCCCAAGATGGCAACACTTTAT 1060
N Y K N V V I F G N R Q A D R S P C G T G T S A K M A T L Y 342
GCCAAAGGCCAGCTTCGCATCGGAGAGACTTTTGTGTACGAGAGCATACTCGGCTCACTCTCCAGGCCAGGGTACTTGGGGAGGAGCCGA 1150
A K G Q L R I G E T F V Y E S I L G S L F Q G R V L G E E R 372
ATACCGGGGTGAAGTGGCGGTGACCAAGATGCCAGGAAGGATGCTCGTTGTAACGGCAGAAATTACTGGAAGGCTTTTATCATG 1240
I P G V K V P V T K D A E E G M L V V T A E I T G K A F I M 402
GGTTTCAACACCATGCTGTTGACCCCAACGGATCCGTTTAAGAACGGATTACATTAAGCAGTAGATCTGGTAGAGCACAGAACTATT 1330
G F N T M L F D P T D P F K N G F T L K Q 423
GGGGAACACGTGCGAACAGGTGCTGTACGTGAAGGTATTGAATGAATCGTTTTTTTATTTTATTTTATTTTATTTATTTAGTGCATT 1420

ATTATTAAATTTTTTTTTTTGGGGTTTCAACGGTACC GGTTGGAGCAGGAAGCGATAGCGGCCGGACAATTTTTCGTTTTAT 1510
TTTCATTTTCATCTTCCTACCCAAACCCCTTGTTCCACCGGTCCGGCGGGGTCTTGTTGGGTGGAGGAGTCCTAAATCCCGCACCTCGG 1600

AGGAATAACATATTTCATATTTCATATCTTGAATCAAAGGCAT 1651

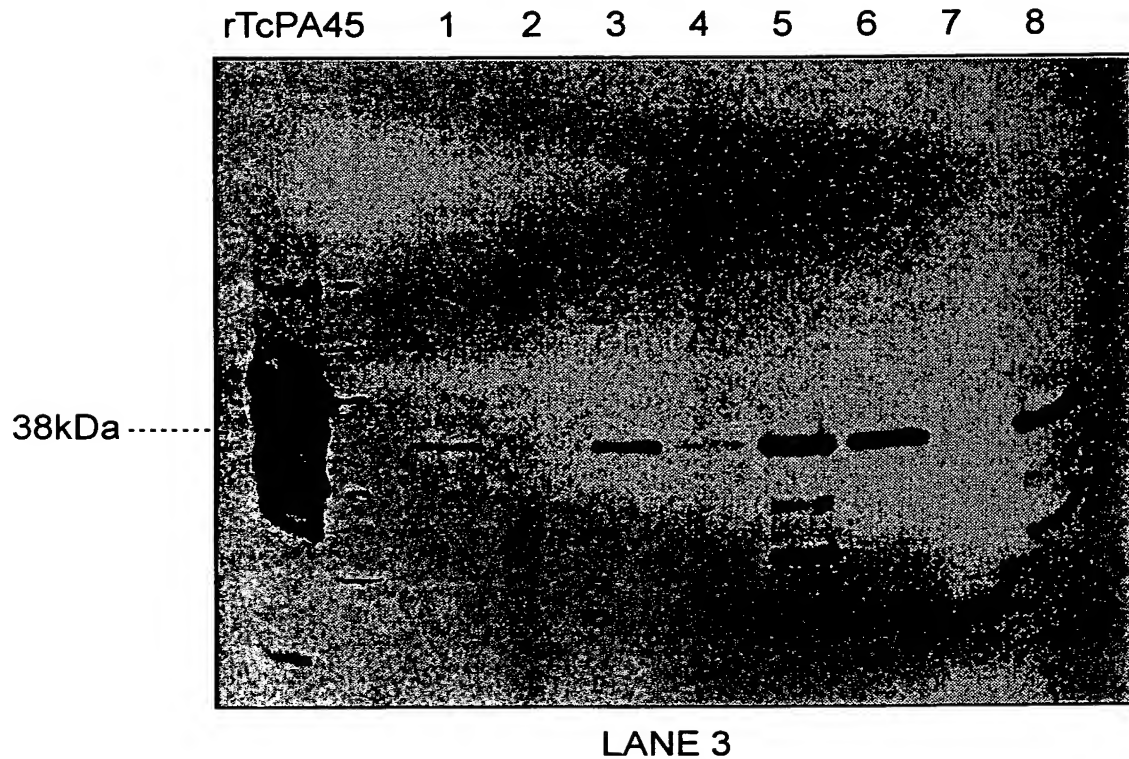
POLYADENILATION SITE

OBS: UNDERLINED THE SEQUENCED PEPTIDES USED TO DEDUCE DEGENERATED PRIMERS FOR CLONING

NUCLEOTIDE SEQUENCE AND PEPTIDE SEQUENCE TcPA45

FIG. 5B

WESTERN BLOT



SOLUBLE FRACTION OF EPIMASTIGOTES EXTRACT (CYTOSOLIC)
REVEALED WITH ANTIBODY DIRECTED TO rTcPA45

----- DEMONSTRATES THE EXISTANCE OF AN INTRACYTOPLASMIC
FORM OF TcPA45 IN THE PARASITE

FIG. 6

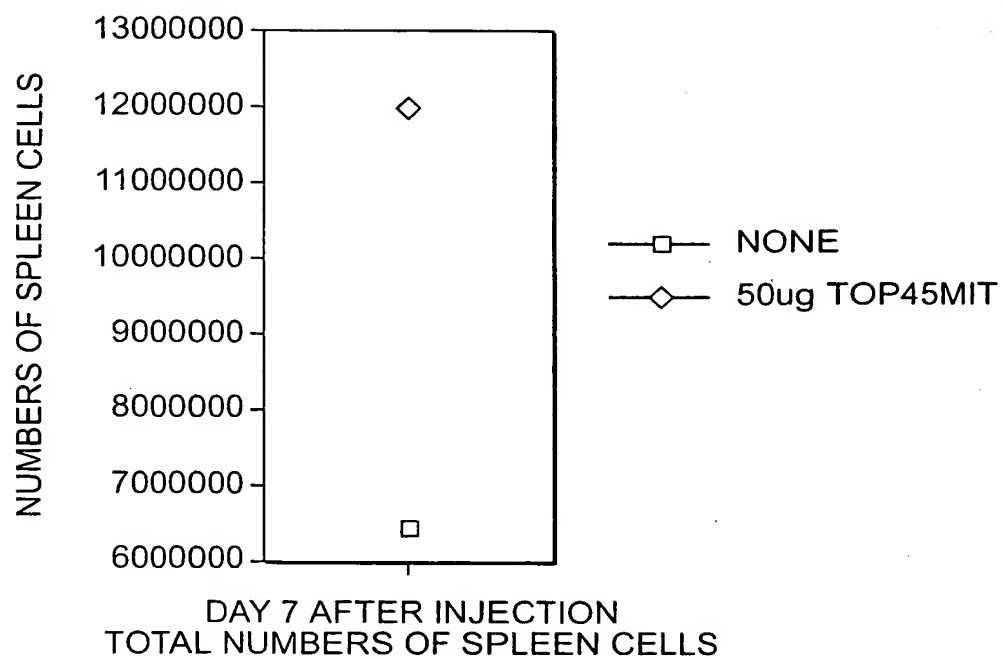


FIG. 7

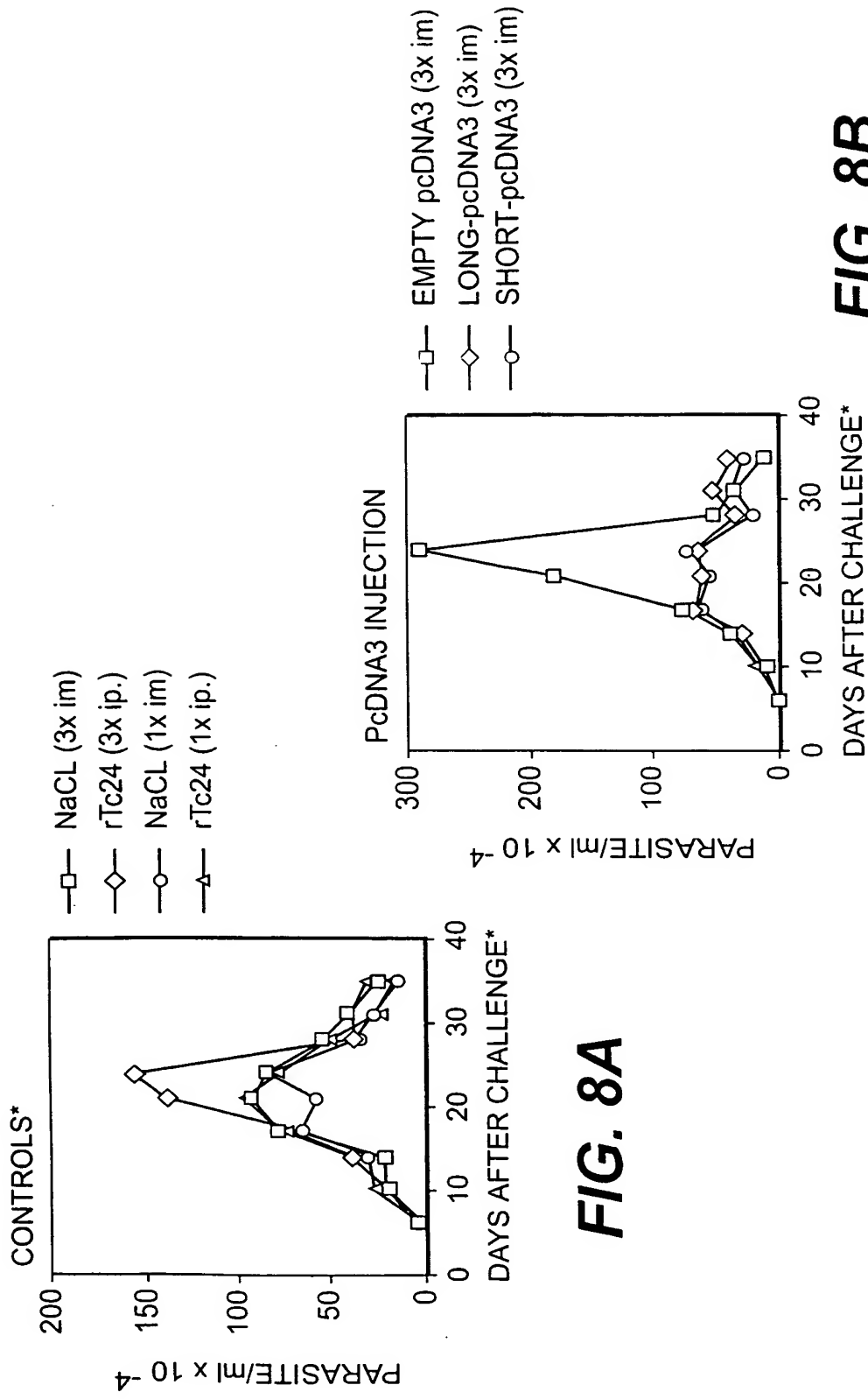


FIG. 8A

FIG. 8B

- | | | | | | |
|-----|------------------------|-----|------------------------|-----|------------------------|
| —□— | EMPTY pcDNA3 (1x i.m.) | —□— | EMPTY VR 1020 (3 i.m.) | —□— | EMPTY VR 1020 (1 i.m.) |
| —◇— | LONG pcDNA3 (1x i.m.) | —◇— | LONG VR 1020 (3 i.m.) | —◇— | LONG VR 1020 (1 i.m.) |
| —○— | SHORT pcDNA3 (1x i.m.) | —○— | SHORT VR 1020 (3 i.m.) | —○— | SHORT VR 1020 (1 i.m.) |

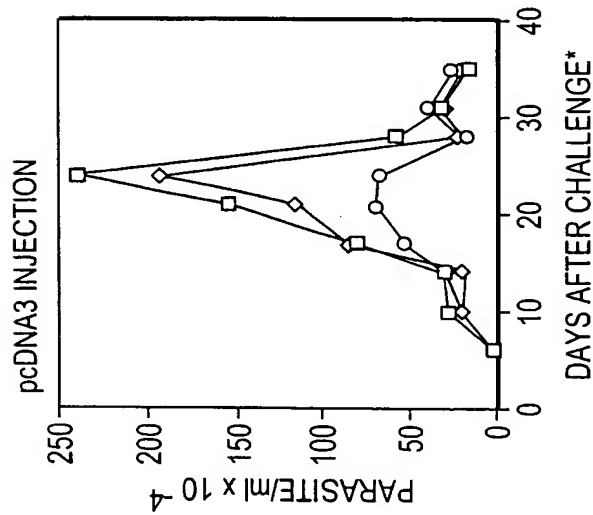


FIG. 8C

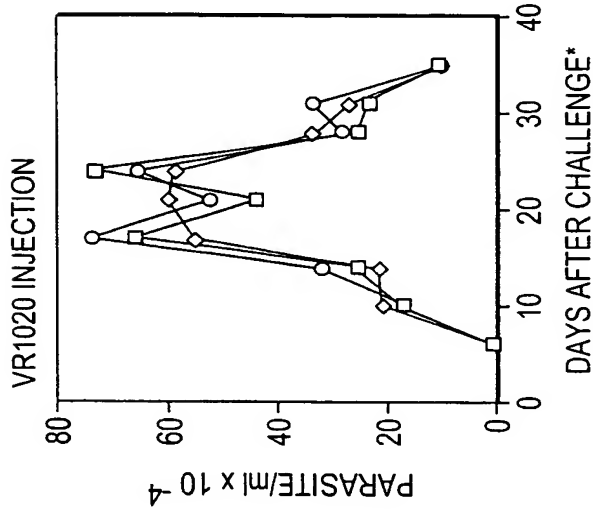


FIG. 8D

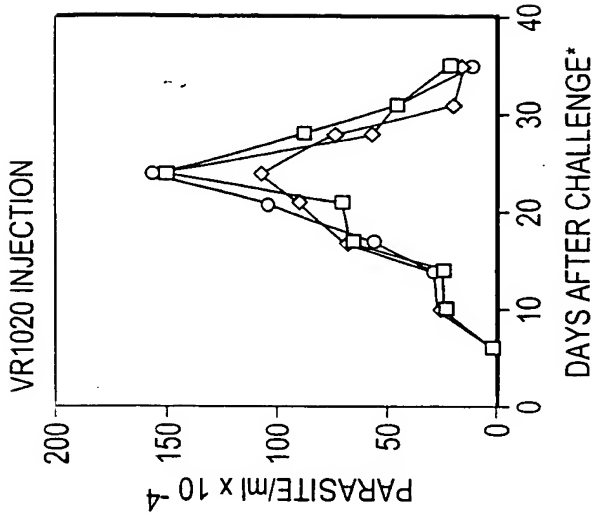


FIG. 8E

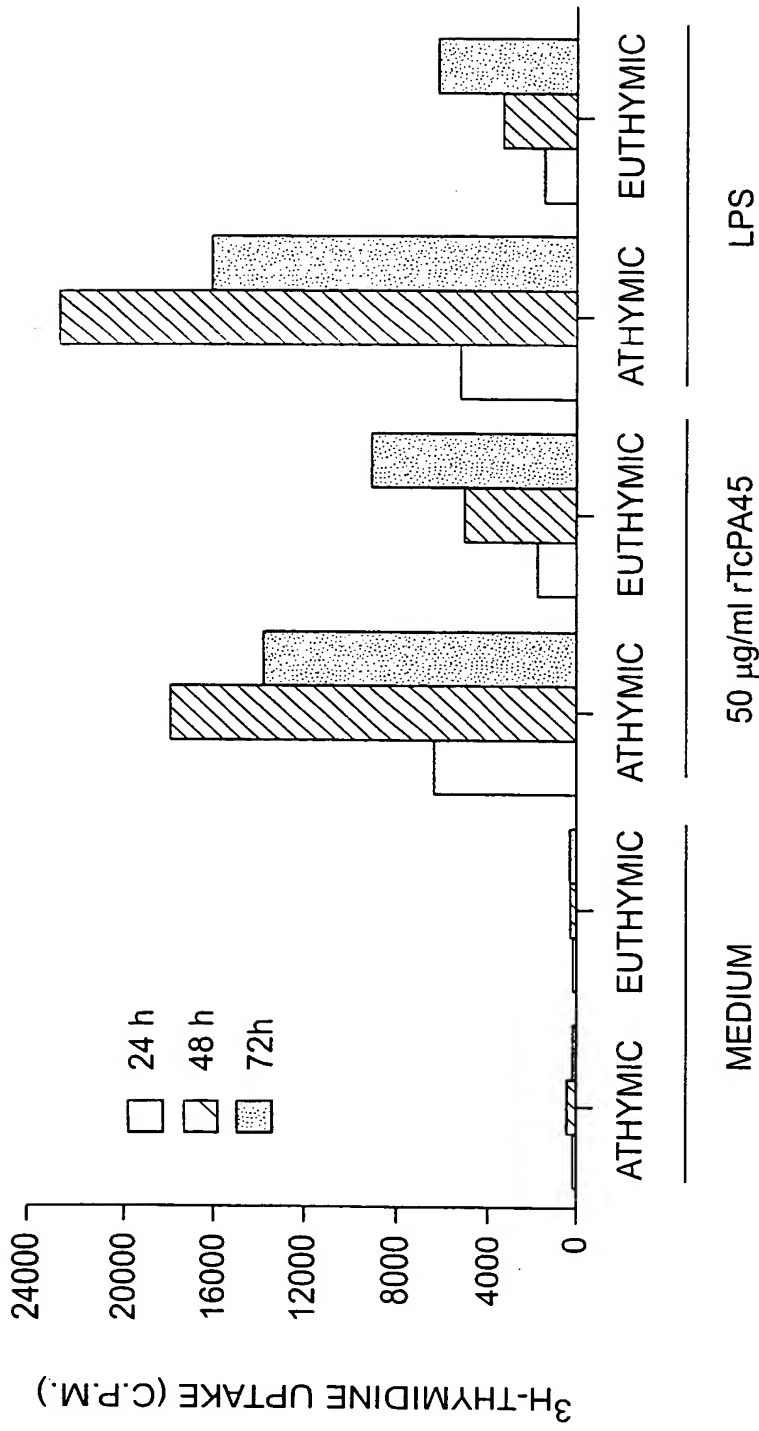


FIG. 9

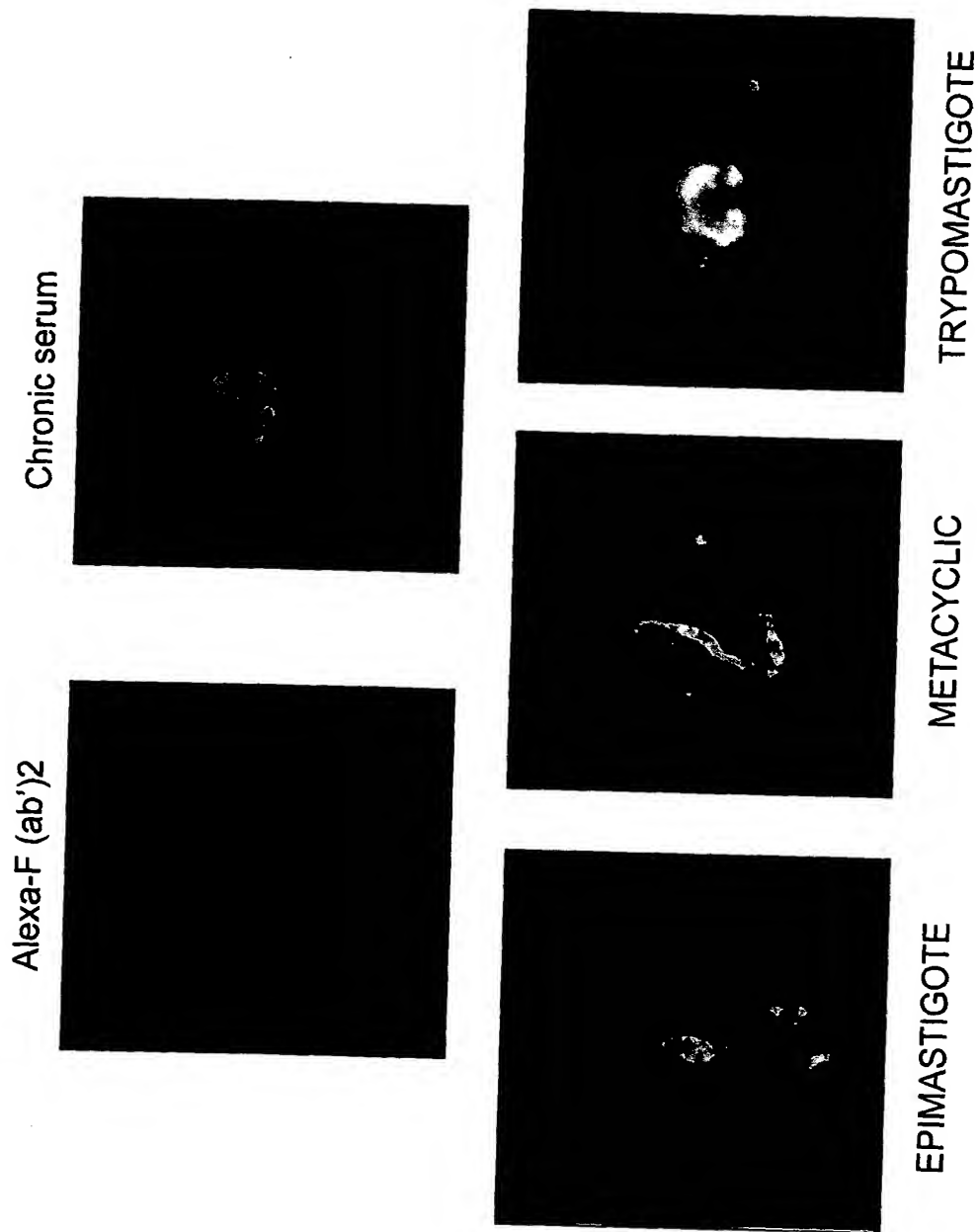


FIG. 10A

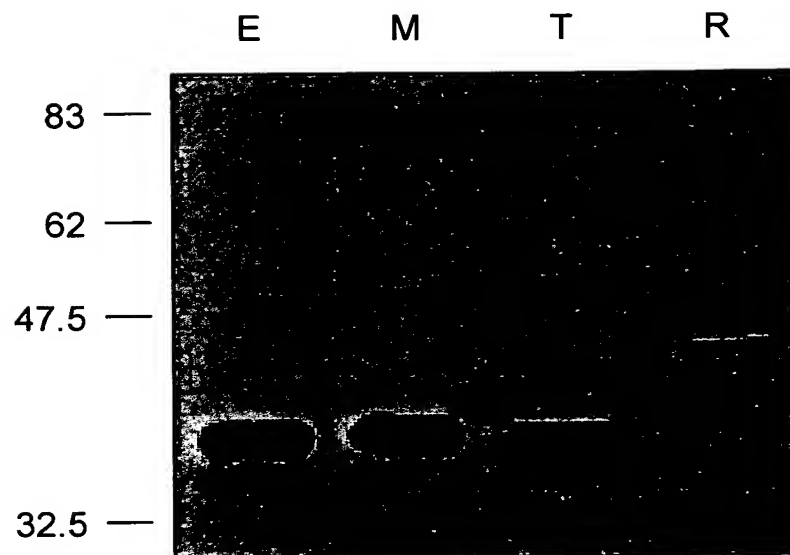


FIG. 10B

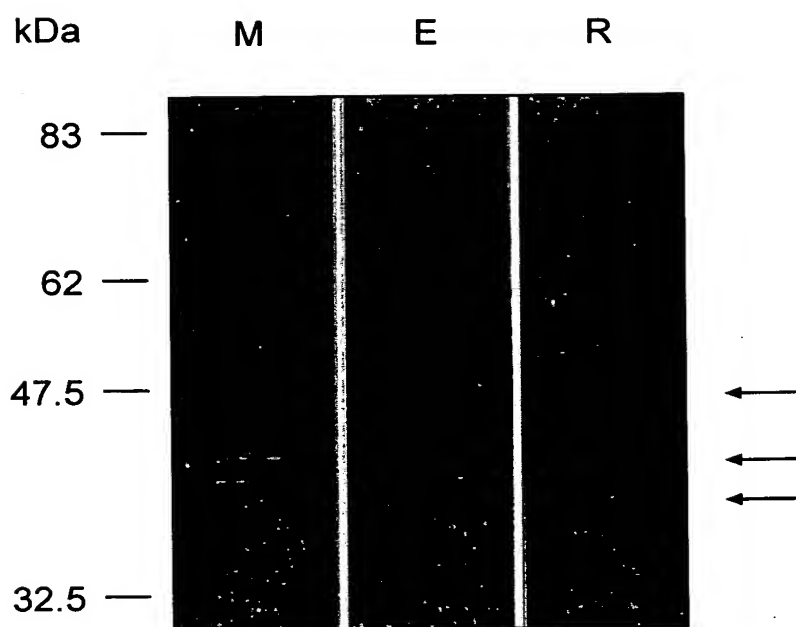


FIG. 10C

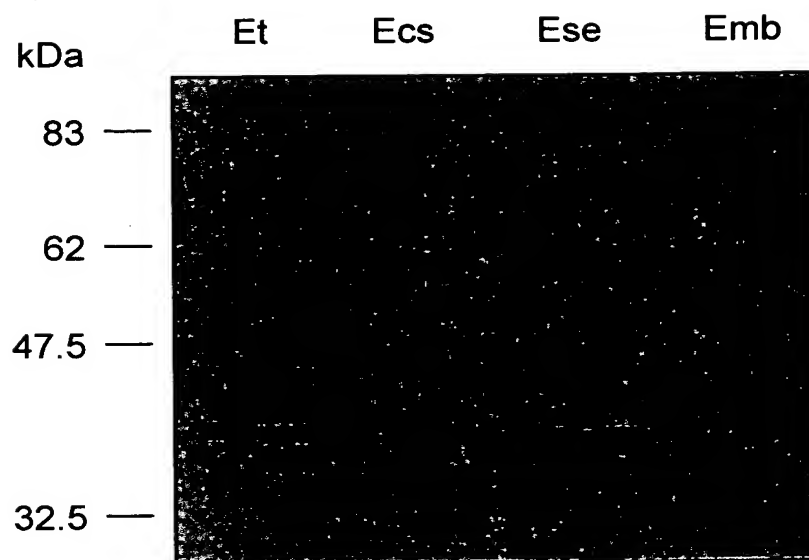


FIG. 10D

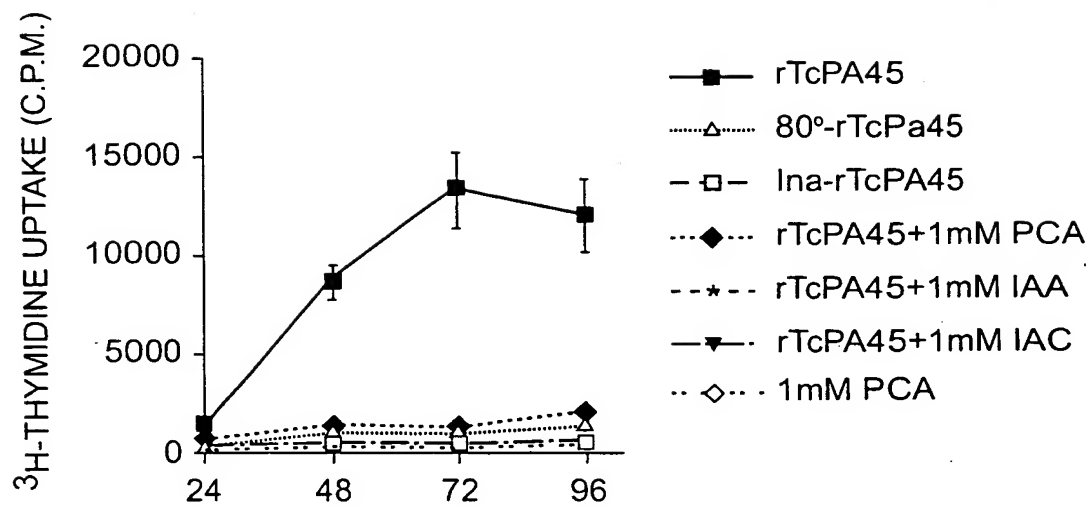


FIG. 11A

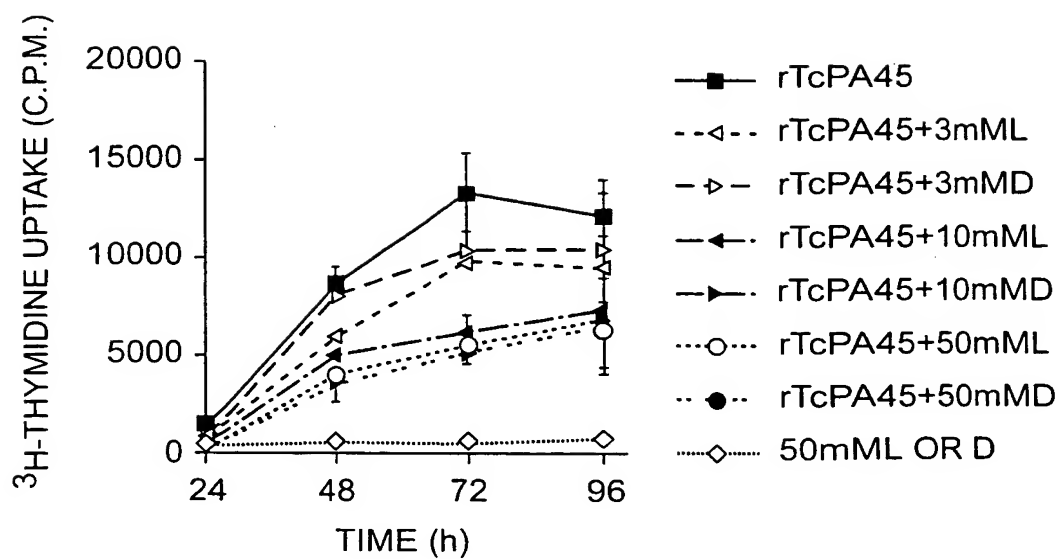


FIG. 11B

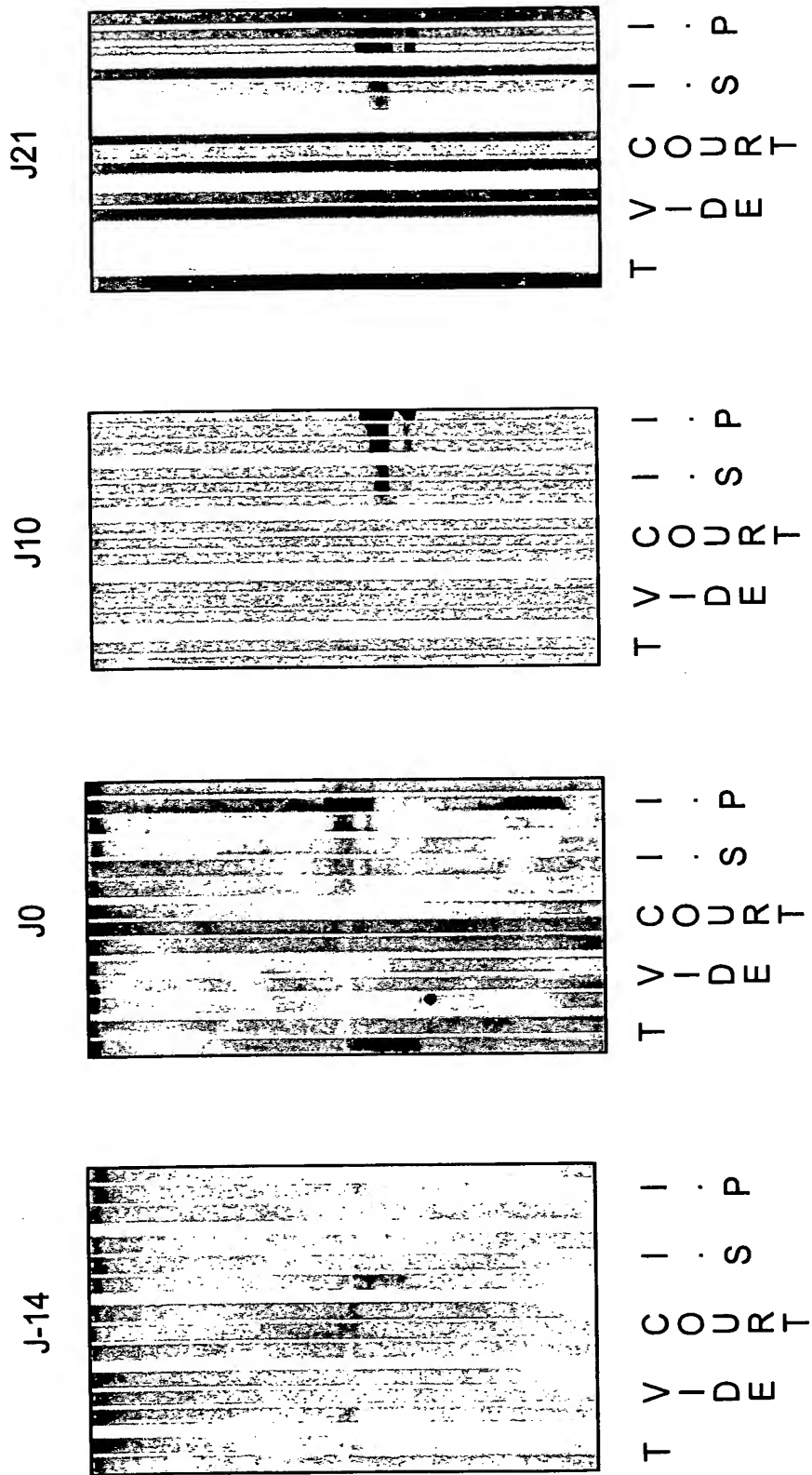


FIG. 12

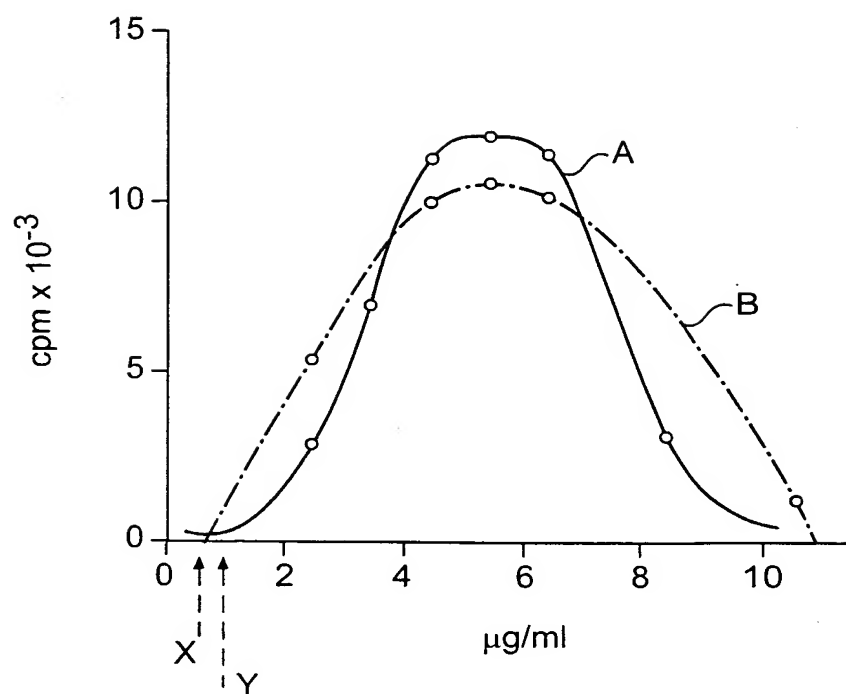


FIG. 13

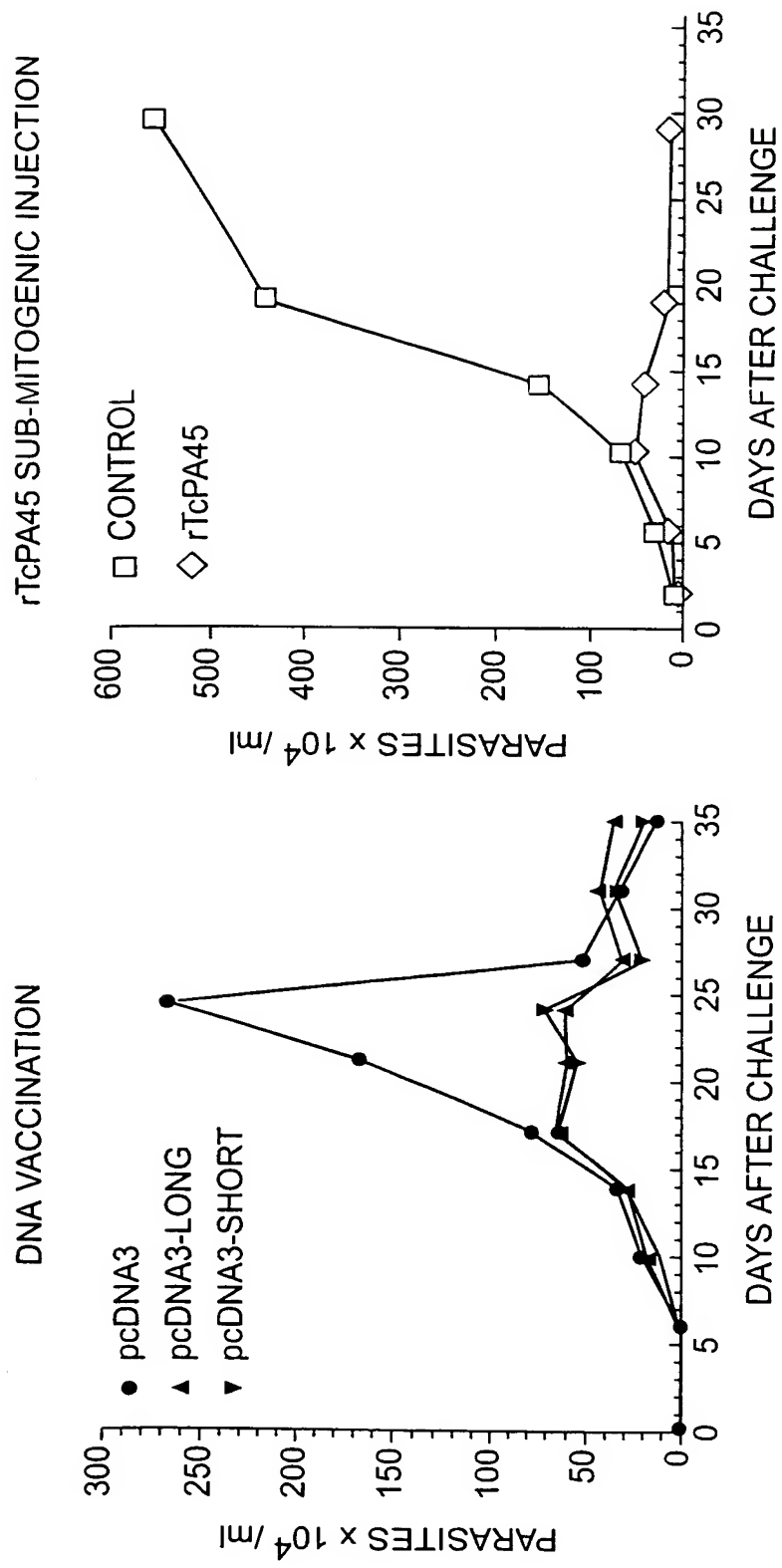


FIG. 14A

FIG. 14B

SEQ ID NO:2

Tc	RTGQEKLLFDQYKILIKGEKKKKKNQRANRREHQQKREIMRFKKS	75
Tc	FTCIDMHTEGEAARIVTSGLPHPGSGNMAEKKAYLQENMDYLRRGIMLEPRGHDDMFGAFLFDPIEEGADLGMVF	150
Tc	MDTGGYLNMCGHNSIAAVTAAVETGIVSVPAKATNPVVLDTPAGLVRGTAHLQSGTESEVSNASIIINVPSFLYQ	225
Tc	QDVVVVLPKPYGEVRVDIAFGGNFFAIVPAEQIGIDISVQNL SRLQEAGELLRTEINRSVKVQHPQLPHINTVDC	300
Tc	VEIYGPPTNPEANYKMVVIFGNRQADR SPCGT GTSAKMATLYAKGQLRIGETFVYESILGSLFQGRV--LGEE	371
Tc	RIPGVKVPVTKDAEEGMLVVTAETGKAFIMGFNTMLFDPTDPFKNGFTLKQ*	423

FIG. 15

SEQ ID NO:4

Tc		MRFKKS	75
Tc	FTCIDMHTEGEAA <u>RI</u> VTSGLP <u>HI</u> PGSNMAEKKAYLQENMDYLRRGIMLEPRGHDDMFGAFLFDPIEEGADLG	MVF	150
Tc	MDTGGYLNMCCHNSIAAVTAAVETGIVSVPAKATNPVVLDT <u>PAGLV</u> RGTAHLQSGTESEVSNASIIINVPSFLYQ		225
Tc	QDVVVLPKPYGEVR <u>VDIA</u> FGGNFFAIVPAEQLGIDISVQNLSRLQEAAGELLRTEINRSVKVQHPQLPHINTVDC		300
Tc	VEIYGPPTNPEANYK <u>NVVI</u> FGNRQADR SPCGT GTSAKMATLYAKGQLRIGETFVYESILGSLFQGRV--LGEE		371
Tc	RIPGVKVPVTKDAEEGMLVVVTAEITGKAFIMGFNTMLFDPTDPFKNGFTLKQ*		423

FIG. 16

SEQ ID NO:7

POLYPYRIMIDINE RICH REGION



SPLICE LEADER
ACCEPTOR SITES

SIGNAL PEPTIDE

CCTTTTCTTTTAAACAAAAATTCGGGGGGAATATGGAACAGGGTATATGCGTAAAGTGCTGTCCCAACAAAAATTTT 90
M R K S V C P K Q K F F 12
TTTTCCGCCCTCCCATTTTTTTTTTTTGTGTGTTTCCCTTGATCTCTCGAACAGGCAGGAAAGCTTCTGTTCACCAAAAAATAT 180
F S A F P F F F F C V F P L I S R T G Q E K L L F D Q K Y 42
AAATTTAAGGGCGAGAAAAAGAAAAATCAACGAGCAACAGGAGAGAACCAACAAAAAGGAAATTTATCGGATTT 270
K I I K G E K K E K K N Q R A N R R E K Q Q K R E I M R F 72
AAGAAATCATTACATCGACATGCATACGGAAGGTGAACGACGCGGATTGTGACGAGTGGTTGCCACACATTCAGGTTCGAAT 360
K K S F T C I D M H T E G E A A R I V T S G L P H I P G S N 102
ATGGCGGAGAAAGCATACCTGCAGGAAAAACATGGATTATTGAGGCGTGGCATAATGCTGGAACCCACGTGGTCATGATGATATGTTT 430
M A E K A Y L Q E N M D Y L R R G I M L E P R G K D D M F 132
GGAGCCTTTTATTGACCTATTGAAGAAGCGCTGACTTGGGCAATGGTATTTCATGGATACCGTGGCTATTTAAATATGTGTGGACAT 520
G A F L F D P I E E G A D L G M V F M D T G G Y L N M C G H 162
AACTCAATTGCAGCGGTACGGCGGCAGTTGAAACGGGAATTGTAGCGTGCCGCGAAGGCAACAAATGTTCCGGTTGTCTCGGACACA 610
N S I A A V T A A V E T G I V S V P A K A T N V P V L D T 192
CCTGCGGGGTGGTCCCGGTACGGCACACCTTCAGAGTGGTACTGAGAGTGAGGTGTCAAATGCGAGTATTATCAATGATCCCTCATTT 700
P A G L V R G T A R L Q S G T E S E V S N A S I I N V P S F 222
TTGTATCAGCAGGATGTGTGTTGTGTGCCAAAGCCCTATGTTGAAGTACGGGTGATATTGCATTTGGAGGCAATTTTTCGCCCAT 790
L Y Q Q D V V V V L P K P Y G E V R V D I A F G G N F F A I 252

FIG. 17A

GTTCCTCCGGAGCAGTTGGGAATTGATAATCTCCGTTCAAAACCTCTCCAGGCTGCAGGAGGCAGGAACTTCTGCGTACTGAAATCAAT 880
V P A E Q L G I D I S V Q N L S R L Q E A G E L L R T E I N 282
CGCAGTGTGAAGGTTACGACCCCTCAGCTGCCCCATATTAAACACTGTGGACTGTGTGAGATATACGCTCCGCCCAACGAACCCGGAGGCA 970
R S V K V Q H P Q L P H I N T V D C V E I Y G P P T N P E A 312
AACTACAAGAACGTTGTGATATTGGCAATCGCCAGGGGATCGCTCTCCATGTGGACAGGCACCAGCGCCCAAGATGGCAACACTTTAT 1060
N Y K N V V I F G N R Q A D R S P C G T G T S A K M A T L Y 342
GCCAAAGGCCAGCTTCGCATCGGAGAGACTTTTGTACGAGAGCATACTCGGCTCACTCTTCCAGGCGAGGTACTTGGGAGGAGCGCA 1150
A K G Q L R I G E T F V Y E S I L G S L F Q G R V L G E E R 372
ATACCGGGGTGAAGGTCCCGTGACCAAAAGATCCGAGGAAGGATGCTCGTTGTAACGGCAGAAATTACTGGAAGGCTTTTATCATG 1240
I P G V K V P V T K D A E E G M L V V T A E I T G K A F I M 402
GGTTTCAACACCATGCTGTTGACCCCAACGGATCCGTTTAAGAACGGATTTCACATTAAAGCAGTAGATCTGGTAGAGCACAGAAACTATT 1330
G F N T M L F D P T D P F K N G F T L K Q * 423
GGGGAACACGTGCGAACAGGTGCTGCTACGTGAAGGTATTGAATCGTTTTTTTTTTTATTATTTTATTATTAGTGCAAT 1420

ATTATTAAATTTTTTTTTTGGGGTTTCAACGGTACCGGTTGGGAGCAGGGAAGCGATAGCGCGCGGACAAATTTTTTGCCTTTAT 1510

TTTTCATTTTCATCTTCCACCCCAACCCCTTGGTTCCACCGGTCCGCGGGGGTCTTGTGGTGGAGAGTCCCTAAATCCCGCACCTCGG 1600

AGGAATAAACATATTTCAAATTTTCATATCTTGGAATCAAAAGGCAT 1651

POLYADENILATION SITE

Obs: UNDERLINED THE SEQUENCED PEPTIDES USED TO DEDUCE DEGENERATED PRIMERS
FOR CLONING

(b) NUCLEOTIDE SEQUENCE AND PEPTIDE SEQUENCE TcPA45

FIG. 17B

FIG. 18A

AACTACAAGAACGTTGTGATATTTGGCAATCGCCAGGGGATCGCTCTCCATGTGGGACAGGCACCAGCGCCAAGATGGCAACACTTTAT 1060
N Y K N V V I F G N R Q A D R S P C G T G T S A K M A T L Y 342
GCCAAAGGCCAGCTTCGCATCGGAGAGACTTTTGTGTACGAGAGCATACTCGGCTCACTTCCAGGGCAGGGTACTTGGGGAGGAGCGA 1150
A K G Q L R I G E T F V Y E S I L G S L F Q G R V L G E E R 372
ATACCGGGGTGAAGGTCCCGTGACCAAGATGCCGAGGAAGGATGCTCGTTGTACGGCAGAAATTACTGGAAGGCTTTTATCATG 1240
I P G V K V P V T K D A E E G M L V V T A E I T G K A F I M 402
GGTTTCAACACCATGCTGTTTGACCCCAACGGATCCGTTTAAGAACGGATTACATTAAAGCAGTAGATCTGGTAGAGCACAGAAACTATT 1330
G F N T M L F D P T D P F K N G F T L K Q * 423
GGGAACACGTGCGAACAGGTGCTGCTACGTGAAGGTATTGAATGAATCGTTTTTTTATTATTTTATTATTTATTATGTCATT 1420

ATTATTAAATTTTTTTTTTGGGTTTCAACGGTACCGCGTTGGGAGCAGGGAAGCATAGCGCCCGGACAAATTTTTTGCTTTTAT 1510

TTTTCATTTTCATCTTCCTACCCCAACCCCTTGGTTCCACCGTCCGGCGGGGTCTGTGGTGGAGAGTCTCTAAATCCCCGACCTCGG 1600

AGGAATAAACATATTTCAATTTTCATATCTTGGAATCAAAAGGCAT 1651

POLYADENILATION SITE

Obs: UNDERLINED THE SEQUENCED PEPTIDES USED TO DEDUCE DEGENERATED PRIMERS
FOR CLONING

NUCLEOTIDE SEQUENCE AND PEPTIDE SEQUENCE TcPA45

FIG. 18B

SEQ ID NO:9

CGAACAGGGCAGGAAAAGCTTCTGTGTGACCAAAATAT 270
R T G Q E K L L F D Q K Y 72
AAAATTATTAGGGCGAGAAAAGAAAATCAACGAGCAACAGGAGAACCAACAAAAGGAAATATTATGCGATTT 360
K I I K G E K K E K K N Q R A N R R E H Q Q K R E I M R F 102
AAGAAATCATTCACATCGACATGCATACGGAAGGTGAACGAGCAGCGGATTGTGACGAGTGGTTTGCCACACATTCAGGTTCGAAT 430
K K S F T C I D M H T E G E A A R I V T S G L P H I P G S N 132
ATGGCGGAGAAAGCATACCTGCAGGAAAACATGGATTATTGAGGCGTGGCATAATCTGGAACCCACGTGGTCATGATGATATGTTT 520
M A E K K A Y L Q E N M D Y L R R G I M L E P R G H D D M F 162
GGAGCCCTTTTATTGACCCCTATTGAAGAAGCGCTGACTTGGGCATGGTATTTCATGGATACCGGTGGCTATTTAAATATGTGTGGACAT 610
G A F L F D P I E E G A D L G M V F M D T G G Y L N M C G H 192
AACTCAATTGCAGCGGTTACGGCGGCAGTTGAAACGGGAATTGTAGCGTGCCGCGAAGGCAACAATGTTCCGGTTGTCTCTGGACACA 700
N S I A A V T A A V E T G I V S V P A K A T N V P V L D T 222
CCTGCGGGGTTGGTCCGCGGTACGGCACACCTTCAGAGTGGTACTGAGAGTGAGGTGTCAAATCGGAGTATTATCAATGTACCCCTCATTT 790
P A G L V R G T A H L Q S G T E S E V S N A S I I N V P S F 252
TTGTATCAGCAGGATGTGGTGGTTGTGTGCCAAAGCCCTATGGTGAAGTACGGGTTGATATTGCATTTGGAGGCCAATTTTTCGCCCATTT 880
L Y Q Q D V V V V L P K P Y G E V R V D I A F G G N F A I 282
GTTCCCGCGGAGCAGTTGGGAATTGATATCTCCGTTCAAAACCTCTCCAGGCTGCAGGAGCAGGAGAACTTCTGCGTACTGAAATCAAT 970
V P A E Q L G I D I S V Q N L S R L Q E A G E L L R T E I N 312
CGCAGTGTGAAGGTTCAGCACCTCAGCTGCCCCATATTAACTGTGGACTGTGTGAGATATACGGTCCGCCAACGAACCCGGAGGCA 1060
R S V K V Q H P Q L P H I N T V D C V E I Y G P P T N P E A 342
AACTACAAGAACGTTGTGATATTGTGGCAATCGCCAGCGCGGATCGCTCTCCATGTGGGACAGGCACCGCCCAAGATGGCAACACTTTAT 1150
N Y K N V V I F G N R Q A D R S P C G T G T S A K M A T L Y 372

FIG. 19A

POLYADENYLATION SITE

NUCLEOTIDE SEQUENCE AND PEPTIDE SEQUENCE TcPA45

FIG. 19B

SEQ ID NO:10

SIGNAL PEPTIDE

ATGCCGTAAAGTGTCGTCCCAACAAATTTT

TTTCCGCCCTCCCATTTTTTTTTTTTGTGTGTTCCCTTGATCTCT

NUCLEOTIDE SEQUENCE OF SIGNAL SEQUENCE TcPA45

FIG. 20

SEQ ID NO:11

AAAGAAATCATTACATGCGATCGACATGCATACGGAAGGTGAAGCAGCAGCGGATTGTGACGAGTGGTTGCCACACATTCAGGTTCAAT 360
K K S F T C I D M H T E G E A A R I V T S G L P H I P G S N 102
ATGGCGGAGAGAAAGCATACCTGCAGGAAAACATGGATTATTTCAGGCGTGGCATAATGCTGGAACCCACGTTGGTCATGATATGTTT 430
M A E K K A Y L Q E N M D Y L R R G I M L E P R G H D M F 132
GGAGCCTTTTATTGACCCCTATTGAAGAAGCGCTGACTTGGGCATGGTATTTCATGGATACCGGTGGCTATTTAAATATGTGTGGACAT 520
G A F L F D P I E E G A D L G M V F M D T G G Y L N M C G H 162
AACTCAATTGCAGCGGTTACGGCGGCAGTTGAAACGGGAATTGTGAGCGTGCCGGGAAGGCAACAATGTTCCGGTTGTCTGGACACA 610
N S I A A V T A A V E T G I V S V P A K A T N V P V L D T 192
CCTGCGGGGTTGGTCCGGGTACGGCACACCTTCAGAGTGGTACTGAGAGTGAGGTGTCAAAATGCGAGTATTATCAATGTACCCCTCATTT 700
P A G L V R G T A H L Q S G T E S E V S N A S I I N V P S F 222
TTGTATCAGCAGGATGTGGTGTGTGTCCAAAGCCCTATGGTGAAGTACGGGTGATATTGCATTTGGAGGCAATTTTTCGCCCAT 790
L Y Q Q D V V V L P K P Y G E V R V D I A F G G N F A I 252
GTTCCCGCGGAGCAGTTGGGAATTGATATCTCCGTTCAAACCTCTCCAGGCTGCAGGAGGCGAGGAGAACTTCTGCGTACTGAAATCAAT 880
V P A E Q L G I D I S V Q N L S R L Q E A G E L L R T E I N 282
CGCAGTGTGAAGGTTCAGCACCCCTCAGCTGCCCCATATTAAACACTGTGGACTGTGTTGAGATATACGGTCCGCCAACGAACCCGGAGGCA 970
R S V K V Q H P Q L P H I N T V D C V E I Y G P P T N P E A 312
AACTACAAGAACGTTGTGATATTGGCAATCGCCAGGCGGATCGCTCTCCATGTGGACAGGCACCAGCGCCAAGATGGCAACACTTTAT 1060
N Y K N V V I F G N R Q A D R S P C G T G T S A K M A T L Y 342
GCCAAAGGCCAGCTTCGCATCGGAGAGACTTTTGTGTACGAGAGCATACTCGGCTCACTCTTCCAGGGCAGGTACTTGGGAGGAGCGA 1150
A K G Q L R I G E T F V Y E S I L G S L F Q G R V L G E E R 1240
402

FIG. 21A

ATACCGGGGTGAAGTGCCGGTGACCAAGAATGCCGAGGAAGGATGCTCGTTGTACGGCAGAAATTACTGGAAGGCTTTTATCATG 1330
I P G V K V P V T K D A E E G M L V V T A E I T G K A F I M 423
GGTTCAACACCATGCTGTTTGACCCCAACGGATCCGTTTAAGAACGGATTACACATTAAAGCAGTAGATCTGGTAGAGCACAGAAACTATT 1420
G F N T M L F D P T D P F K N G F T L K Q *
GGGGAACACGTGCGAACACAGGTGCTGCTACGTGAAGGGTATTGAATGAATCGTTTTTTTATTATTATTTTATTATTAGTGCATT 1510
ATTATTAAATTTTTTTTTTGTGTTGGGGTTTCAACGGTACCGCGTTGGGAGCAGGGAAGCGATAGCGGCCGGACAATTTTTTGCCTTTTAT 1600
TTTCATTTTCACTTCCTACCCAAACCCCTTGGTTCCACCGGTCGCGGGGGGGTCTTGTGGGTGGAGAGTCCATAATCCCGCACCTCGG 1651
AGGAATAAACATATTCAATTCATATCTTGGAATCAAAAGGCAT

FIG. 21B